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Biochemistry

Direct biochemical mapping of eukaryotic viral DNA by means of a linked transcription-translation cell-free system

(restriction endonucleases/simian virus 40 DNA fragments/wheat germ RNA nucleotidyltransferase II/wheat germ extracts/protein analysis)

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ABSTRACT **A method is described for mapping regions of eukaryotic viral DNA coding for specific proteins, utilizing a linked transcription-translation cell-free system primed with DNA fragments generated by restriction endonucleases.**

Three simian virus 40 (SV40) DNA fragments derived from that region of the DNA expressed late in lytic infection were purified. They were: *Hpa* I-A (0.76–0.175 map units), *Bgl* I-*Eco*RI-B (0.672–0), and *Hpa* II-*Eco*RI-B (0.735–0). (Fragments are named from the cleaving restriction endonuclease and electrophoretic mobility. End positions on the conventional map are in clockwise order.) These fragments efficiently stimulated the incorporation of [^{3}H]UTP and [^{35}S]methionine into trichloroacetic-acid-insoluble material in the linked system. The location of the region of DNA coding for the viral structural proteins VP1, VP2, and VP3 was determined from the spectrum of polypeptide synthesis directed by the individual intact fragments and their specific endonucleolytic digests. The polypeptides synthesized in the cell-free system were characterized on urea-sodium dodecyl sulfate polyacrylamide gradient gels and by two-dimensional tryptic peptide analysis. From this data, it was concluded that the region of SV40 DNA encoding the information for VP1 is between 0.835 and 0.175, VP2 is between 0.76 and 0, and VP3 is between 0.835 and 0 map units. Utilizing this mapping data, comparisons of the tryptic digests, and the coding capacities of the respective DNA fragments, we located the regions coding for the three structural proteins relative to one another on the physical map of SV40 DNA. It is noteworthy that VP2 and VP3 are probably coded for by overlapping DNA sequences.

The precise localization of regions within a genome encoding the information for specific proteins is invaluable for the understanding of eukaryotic DNA organization. Most studies directed towards this goal have utilized the genetic analyses developed in prokaryotes (1–4) and have been successful in the partial mapping of eukaryotic viral functions (5–7) and those of a few eukaryotes (8–10).

The mapping of one animal virus genome, simian virus 40 (SV40) DNA, has been dependent upon the generation of a physical map of the DNA by means of restriction endonucleases (11). Several pertinent features have been defined on the physical map, and they include the origin of DNA replication (12, 13) and the regions of DNA transcribed either early or late during lytic infection (14). In addition, using the separated strands of SV40 DNA, it has been demonstrated that the RNAs found in the cytoplasm during the early and late periods of infection are derived from different DNA strands (15). Complementation analyses of SV40 have defined several complementation groups (16, 17); however, their direct correlation with specific polypeptides has proved difficult.

Abbreviations: SV40, simian virus 40; DNA FI, DNA Form I (supercoiled, closed circular); DNA FIII, DNA Form III (unit-length linear); VP, virus protein; $NaDodSO_4$; sodium dodecyl sulfate.

An early attempt at the biochemical mapping of DNA involved the hybridization of SV40 messenger RNA to SV40 DNA, and the subsequent reisolation and translation of the hybridized messenger RNA (18). This approach was improved and extended to map those regions of adenovirus 2 DNA coding for viral proteins expressed either early or late in infection (19).

In this paper we elaborate on the use of a linked transcription-translation cell-free system to map the regions of SV40 DNA encoding the information for the viral structural proteins VP1, VP2, and VP3.

MATERIALS AND METHODS

Wheat germ was kindly supplied by W. C. Mailnot, General Mills, Inc., Minneapolis, Minn. [^{35}S]Methionine (300 Ci/mmol) was from Amersham and [^{3}H]UTP (40 Ci/mmol) from New England Nuclear Co. The restriction endonucleases *Bam*HI from *Bacillus amyloliquefaciens* H, *Hae* II from *Haemophilus aegyptius*, *Hpa* I and *Hpa* II from *Haemophilus parainfluenzae*, *Hha* I from *Haemophilus haemolyticus* (ATCC 10014), and *Bgl* I from *Bacillus globiggi* were generously provided by R. J. Roberts. *Eco*RI was purified from *Escherichia coli* strain RY13 carrying the RI drug resistance plasmid (20). Wheat germ polymerase II (RNA nucleotidyltransferase) was kindly provided by Jerry Jendrisak and prepared by the procedure of Jendrisak and Burgess (21). Adenovirus 2 DNA was a kind gift of Jane Flint, and phage T4 DNA ligase was kindly supplied by Dan Donaghue.

Cells and Virus. A plaque-purified stock of SV40 (strain 777) was grown in the BSC-1 line of African green monkey kidney cells and [^{35}S]methionine-labeled virus was prepared and purified (22). Closed circular viral DNA Form I (FI) was prepared by the method of Hirt (23). Ethidium bromide was removed by passage of the DNA solution through a Dowex 50 column equilibrated with 10 mM Tris·HCl at pH 7.4 and 0.1 mM EDTA.

DNA Digestion and Purification of Restriction Fragments of SV40 DNA. SV40 DNA FI and purified restriction fragments were digested to completion with the specified restriction endonucleases in 6 mM Tris·HCl, pH 7.9, 6 mM Mg acetate, 6 mM 2-mercaptoethanol at 37° (24).

DNA fragments were separated preparatively in 1% agarose gels containing 0.5 μg/ml of ethidium bromide (25); bands were visualized briefly by fluorescence with ultraviolet light (Mineralight UVS-12) and excised. The DNA was recovered by the method of Southern (26), modified only in that the hydroxyapatite chromatography was at 37°. Thereafter, phosphate was removed by dialysis against 10 mM Tris, pH 7.4, and 0.1 mM EDTA at 4°, and ethidium bromide was removed by Dowex

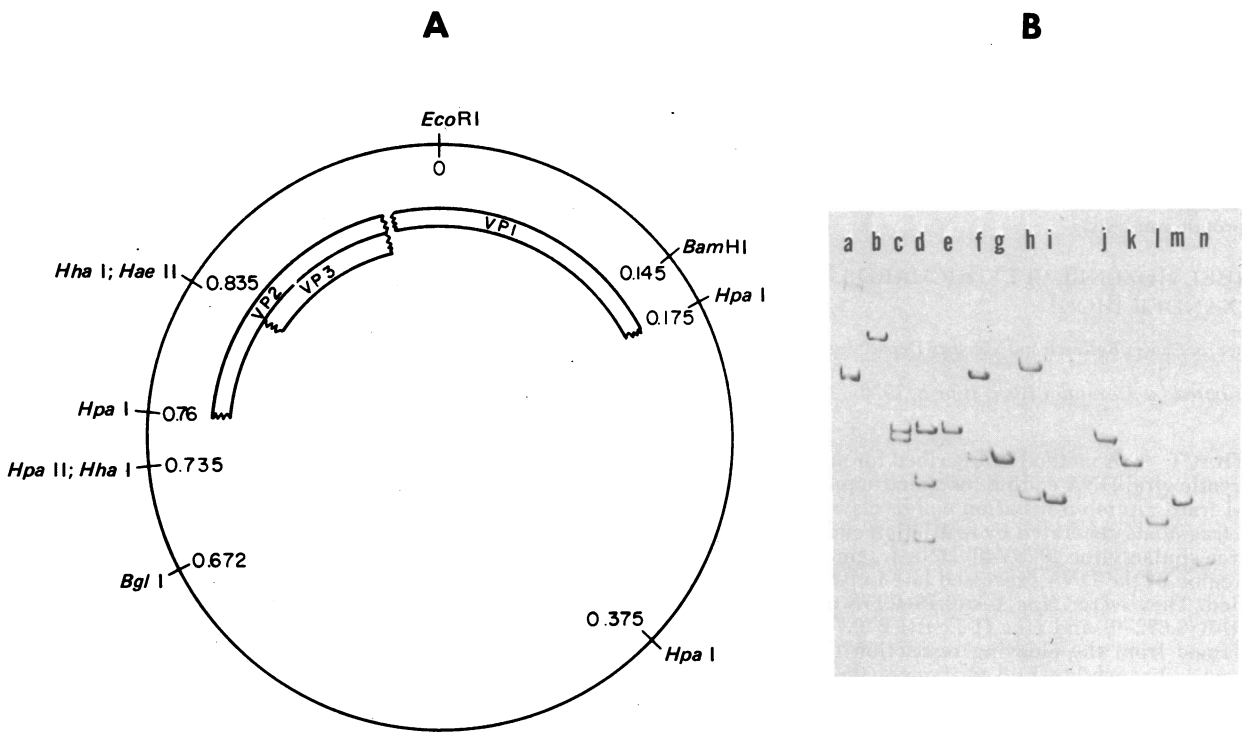

FIG. 1. (A) A map of the SV40 genome. The cleavage site of *Eco*RI endonuclease designates the zero position and the cleavage site(s) of the other endonucleases used in this study are given in relation to total length of SV40 in a clockwise direction. *Hpa* II and *Hha* I are indicated to cleave the DNA at 0.735 since both enzymes cleave different nucleotide sequences that occur very close to this map position. The cleavage positions of the endonucleases were compiled and kindly provided by S. Weissman. The location of the sequences coding for VP1, VP2, and VP3 are rationalized in the discussion.

(B) Agarose gel electrophoresis of restriction fragments of SV40 DNA. The DNA was subjected to electrophoresis in an analytical 1% agarose gel at 50 V for 10 hr and the gel was dried. The fluorescent bands were observed by direct illumination with shortwave ultraviolet light (UV Products, San Gabriel, Calif.) and recorded using Polaroid type 55 P/N film and a Kodak no. 23A red filter with an exposure time of 5 sec.

The samples are: (a) SV40 DNA FI, (b) SV40 DNA FIII, (c) *Hpa* I total digest, (d) *Hpa* I-*Bgl* I total digest, (e) purified *Hpa* I-A (0.76–0.175) fragment, (f) *Bgl* I-*Eco* RI total digest, (g) purified *Bgl* I-*Eco*RI-B (0.672–0) fragment, (h) *Hpa* II-*Eco*RI total digest, (i) purified *Hpa* II-*Eco*RI-B (0.735–0) fragment, (j) *Hpa* I-A (0.76–0.175) fragment, (k) *Hha* I digest of the *Hpa* I-A (0.76–0.175), (l) *Eco*RI digest of the *Hpa* I-A (0.76–0.175) fragment, (m) *Hpa* II-*Eco*RI-B (0.735–0), (n) *Hha* I digest of the *Hpa* II-*Eco*RI-B (0.735–0) fragment.

50 chromatography. Finally, the DNA was precipitated with ethanol in the presence of 0.2 M Na acetate, pH 5.5, the pellet was washed with 60% ethanol, and the DNA was stored in 10 mM Tris·HCl, pH 7.4, and 0.1 mM EDTA at −80°. The recovery of DNA by this method was between 60% and 80%. Three DNA fragments containing sequences known to code for RNAs transcribed late in the lytic cycle were purified. They were: *Hpa* I-A (0.76–0.175 map units); *Bgl* I-*Eco*RI-B (0.672–0), and *Hpa* II-*Eco*RI-B (0.735–0). The position of these fragments on the physical map of SV40 DNA can be located in Fig. 1A. The purity of the isolated fragments was assessed by comparison to the total digests in an analytical 1% agarose gel (Fig. 1B). The mobilities of SV40 DNA, FI, and unit length linear molecules, FIII, were those shown in Fig. 1B, a and b. Fragments A and B of the *Hpa* I digest of SV40 DNA migrate too close to one another to permit facile purification of A (Fig. 1B, c). Therefore, cleavage of the *Hpa* I-B fragment to yield two smaller constituents was effected by digestion with *Bgl* I (Fig. 1B, d). This double digest of SV40 DNA was utilized to purify the *Hpa* I-A fragment (Fig. 1B, e). The *Bgl* I-*Eco*RI digest of SV40 DNA yields two well-separated fragments (Fig. 1B, f), and was used directly to purify the *Bgl* I-*Eco*RI-B constituent (Fig. 1B, g). Similarly, the separation of *Hpa* II-*Eco*RI-A and B fragments (Fig. 1B, h) was adequate to permit the purification of the B fragment (Fig. 1B, i). Some of these purified fragments were further digested by endonucleases that inflicted single cleavages at specific positions. The completely digested DNA was reisolated by phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation.

Linked Transcription-Translation. DNA-dependent RNA-directed protein synthesis was performed under conditions similar to those reported previously (22). The major modification was the replacement of *E. coli* RNA polymerase with wheat germ RNA polymerase II. The optimal conditions for transcription with the wheat germ enzyme were: *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid 20 mM (Hepes), pH 7.9, 10 mM Mg acetate, 1.0 mM Mn acetate, 0.5 mM for each of the nucleotide triphosphates, 5 mM dithiothreitol, 10–15 μg of wheat germ RNA polymerase II, and 0.5–1 μg of DNA fragment. After 15 min at 37°, the transcription reaction conditions were rendered optimal for protein synthesis (22).

Polyacrylamide Gels. Sodium dodecyl sulfate ($NaDodSO_4$) 10–15% gradient polyacrylamide gels (27) and urea–$NaDodSO_4$ 10–15% gradient polyacrylamide gels (28) were prepared. The gels were run for approximately 6 hr at 200 V (constant voltage), stained with Coomassie blue, impregnated with 2,5-diphenyloxazole (29), dried, and fluorographed at −80°.

Peptide Analysis. The labeled cell-free products were fractionated in urea–$NaDodSO_4$ polyacrylamide gels and the gels stained with Coomassie Brilliant Blue R and dried. The [^{35}S]methionine-labeled polypeptides comigrating with authentic VP1, VP2, and VP3 were located by autoradiography. Purified [^{35}S]methionine-labeled virus was also fractionated

Table 1. Efficiency of templates in the linked system

Template	Incorporation into trichloroacetic-acid-insoluble material	
	pmol of UTP	pmol of methionine
No DNA	38	0.24
SV40 DNA FI	2296	9.26
Hpa I (0.76–0.175)	1596	4.63
Hpa I (0.175–0.375)	979	6.01
Hpa I-*Bgl* I (0.375–0.672)	432	3.60
Hpa I-*Bgl* I (0.672–0.76)	424	2.19
Bgl I-*Eco*RI (0–0.672)	1530	6.65
Bgl I-*Eco*RI (0.672–0)	1596	1.73
Hpa II-*Eco*RI (0–0.735)	1860	7.24
Hpa II-*Eco*RI (0.735–0)	1389	1.44
Adenovirus 2 DNA	1831	3.56

A variety of DNA templates were tested for their ability to stimulate the incorporation of UTP and methionine into trichloroacetic-acid-insoluble material in the linked system. Reactions were performed exactly as described in *Materials and Methods*, except that 1 μCi of [^{3}H]UTP was present in the transcription reaction. 0.5–1.0 μg of each template was used. UTP incorporation was calculated assuming that the fraction of added [^{3}H]UTP incorporated reflected the fraction of unlabeled UTP incorporated. Methionine incorporation was calculated directly from the incorporation of [^{35}S]methionine. This is an underestimate of methionine incorporation, for there is an endogenous pool of methionine in the wheat germ extracts which contributes approximately 2 μM methionine to the final translation conditions.

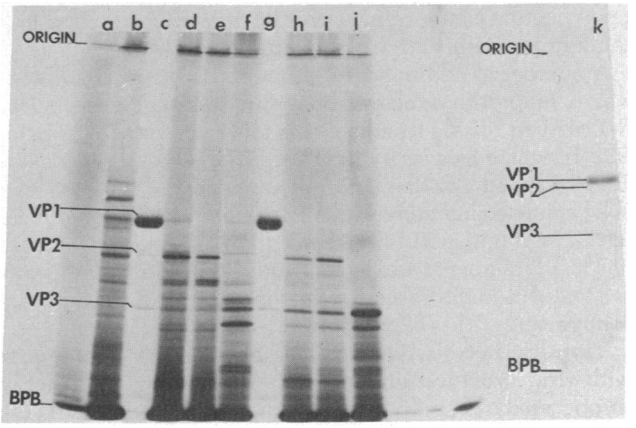

FIG. 2. Fluorograph of [^{35}S]methionine-labeled polypeptides fractionated on gradient polyacrylamide slab gels. The dried gel was fluorographed at −80° for 2 days. Samples a to j were fractionated on a urea–NaDodSO$_4$ 10–15% gradient gel and sample k on a NaDodSO$_4$ 10–15% gradient gel. Apart from the purified virions disrupted and used as markers in c, g, and k, all the samples are [^{35}S]methionine-labeled cell-free products of the linked transcription-translation system primed either with SV40 DNA FI or the specified restriction fragments. The samples were: (a) control without added DNA, (b) SV40 DNA FI, (c) purified virions as marker, (d) *Hpa* I (0.76–0.175) DNA, (e) *Hpa* I (0.76–0.175) DNA cleaved with *Eco*RI at map position 0, (f) *Hpa* I (0.76–0.175) DNA cleaved with *Hha* I at map position 0.835, (g) purified virions as marker, (h) *Bgl* I-*Eco*RI (0.672–0) DNA, (i) *Hpa* II-*Eco*RI (0.735–0) DNA, (j) *Hpa* II -*Eco*RI (0.735–0) DNA cleaved with Hha I at map position 0.835, (k) purified virions as a marker. BPB indicates the position of the bromophenol blue marker.

by the same procedure. In each case, the gel pieces corresponding to VP1, VP2, and VP3 were excised and swollen in NaDodSO$_4$ electrophoresis buffer and the respective proteins were eluted into dialysis bags by electrophoresis for 16 hr at 4 mA per sample. The eluted proteins were then dialyzed extensively against distilled water for 24 hr at room temperature and finally lyophilized. The freeze-dried material was washed four times with 0.1 M hydrochloric acid in 90% ethanol and dried under vacuum. The precipitate was shaken at 37° in 0.5 ml of 1% NH_4HCO_3 containing 50 μg of trypsin treated with L-1-tosylamido-2-phenylethylchloromethyl ketone. After incubation for 6 hr, the supernatant was sequentially lyophilized and dissolved in water three times. Finally, the digest was dissolved in 0.05 M pyridine acetate buffer, pH 3.5, at a final concentration of 2000–5000 cpm/μl and fractionated as described in the *text*.

RESULTS

Three purified DNA fragments containing sequences known to code for RNAs transcribed late in the lytic cycle of SV40 were used to map the viral structural proteins. These fragments were: *Hpa* I-A (0.76–0.175), *Bgl* I-*Eco*RI-B (0.672–0), and *Hpa* II-*Eco*RI-B (0.735–0) as shown in Fig. 1A. Each of these fragments efficiently stimulated the incorporation of both [^{3}H]UTP and [^{35}S]methionine into trichloroacetic-acid-insoluble material in the linked transcription-translation cell-free system (Table 1). Furthermore, their template efficiencies were comparable to those of SV40 DNA FI, Adenovirus 2 DNA, and a number of other purified fragments.

The Nature of the Cell-Free Products. The viral polypeptides VP1, VP2, and VP3, fractionated in NaDodSO$_4$ gradient polyacrylamide gels, show little separation of VP1 and VP2 (Fig. 2k). However, in urea–NaDodSO$_4$ gradient gels these three viral polypeptides were well separated (Fig. 2c and g). Their mobilities relative to proteins of known size suggested approximate molecular weights of 47–51,000 for VP1, 37–39,000 for VP2, and 28–31,000 for VP3. In the absence of added DNA the synthesis of [^{35}S]methionine-labeled polypeptides in the linked system was minimal (Fig. 2a). In contrast, SV40 DNA FI directed the synthesis of a range of discrete polypeptides (Fig. 2b).

***Hpa* I Fragment (0.76–0.175).** This *Hpa* I DNA fragment (Fig. 1B, e) directed the synthesis of several prominent polypeptides, three of which comigrate precisely with the viral structural proteins VP1, VP2, and VP3 (Fig. 2d).

In order to locate the regions of DNA coding for the structural proteins relative to one another, the *Hpa* I fragment was digested to completion with *Eco*RI at map position 0 (Fig. 1B, l) and the total digest was used as a template in the linked system. As shown in Fig. 2e, this digested template directed the synthesis of polypeptides comigrating with VP2 and VP3 but no detectable polypeptide comigrating with VP1. Similar results were obtained utilizing the *Hpa* I-A fragment digested with *Bam*HI, which cleaves at map position 0.145 (results not shown). However, the *Hpa* I fragments digested with *Hha* I at map position 0.835 (Fig. 1B, k) directed the synthesis of polypeptides comigrating with VP1 and VP3 but none migrating at the position of VP2 (Fig. 2f).

***Bgl* I-*Eco*RI (0.672–0) and *Hpa* II-*Eco*RI (0.735–0) Fragments.** The *Bgl* I-*Eco*RI (0.672–0) fragment (Fig. 1B, g) directed the synthesis of two major polypeptides which comigrate precisely with VP2 and VP3 (Fig. 2h). There is no detectable polypeptide comigrating with VP1. The *Hpa* II-*Eco*RI (0.735–0) fragment (Fig. 1B, i) directed an identical spectrum of products (Fig. 2i). As in the case of the *Hha* I digest of the *Hpa* I (0.76–0.175) fragment, the *Hpa* II-*Eco*RI-B fragment cleaved with *Hha* I (Fig. 1B, n) did not direct the synthesis of

a polypeptide comigrating with VP2; however, a polypeptide comigrating with VP3 was obvious (Fig. 2j).

A number of additional experiments, to be published elsewhere, support these observations. For example, after the *Hpa* II-*Eco*RI (0.735–0) fragment was digested with the enzyme *Hae* II, which cleaves SV40 DNA at the identical position as *Hha* I at map position 0.835, it directed the synthesis of a polypeptide comigrating with VP3 but not VP2. Further, when the DNA fragments of the *Hha* I digest of the *Hpa* II-*Eco*RI (0.735–0) fragment were ligated with T4 DNA ligase and used as a template in the linked system, the synthesis of a polypeptide comigrating with VP2 was reinstated.

Tryptic Analysis. The cell-free polypeptides comigrating with viral structural proteins were further characterized by tryptic analyses. Individual [^{35}S]methionine-labeled tryptic digests prepared from both the viral proteins VP1, VP2, and VP3 and their respective counterparts synthesized *in vitro* were each fractionated in two dimensions. In each case, the pattern of the digested cell-free polypeptide was very similar to that of the appropriate viral protein (Fig. 3).

Mixtures of the digested cell-free products and the corresponding viral proteins yielded patterns identical to those obtained with either digest alone. Furthermore, mixtures of VP2 and VP3 demonstrate reproducibly a number of tryptic peptides common to both proteins (data not shown).

DISCUSSION

The linked transcription-translation cell-free system was characterized previously and SV40 DNA FI was shown to direct the synthesis of authentic viral proteins (22). Further, a preliminary attempt at mapping the region encoding the information for the major viral structural protein, VP1, was made, utilizing unit-length linear molecules as templates (22). For a number of reasons this approach was inadequate and resulted in the erroneous location of the sequences coding for VP1. We have found that the yield of viral structural polypeptides in the total cell-free product can vary considerably. In addition, it is evident that SV40 DNA FI and particular unit-length molecules preferentially direct the synthesis of polypeptides derived from the early region. For these reasons, this preliminary approach utilizing solely unit-length linear DNA molecules precluded the unequivocal mapping of the region of DNA encoding the information for the viral structural polypeptides.

In this study we utilized three DNA fragments all derived from the late region of SV40 DNA as templates in the linked system. This abolished the contribution of polypeptide synthesis directed by the early region and allowed the straightforward mapping of the viral structural proteins. Wheat germ RNA polymerase II was utilized rather than *E. coli* polymerase to effect transcription. Although qualitatively the spectrum of cell-free products directed by each enzyme was very similar, from a quantitative standpoint, we found it advantageous to utilize the wheat germ polymerase.

The DNA sequence coding for a specific viral protein was located by deriving the smallest restriction fragment that directed the synthesis of the authentic protein(s). Results thus obtained were corroborated by introducing specific endonucleolytic cleavages within the putative coding region and demonstrating that when this DNA was used as a template, that particular polypeptide was eliminated from the spectrum of cell-free products. This would suggest that the coding sequences for that polypeptide traversed those cleavage sites.

The *Hpa* I-A fragment directed the synthesis of all three viral structural proteins, thus locating the sequences coding for them between 0.76 and 0.175 map units. Digestion of this fragment with various restriction endonucleases demonstrated that the sequences coding for VP1 traversed map positions 0 and 0.145 and those for VP2, 0.835. In contrast, the sequences coding for VP3 traversed none of these sites. The results obtained with the *Bgl* I-*Eco*RI (0.672–0) fragment and the *Hpa* II-*Eco*RI (0.735–0) fragment confirmed that the *Eco*RI site was within the region coding for VP1 and refined the map positions of VP2 and VP3 to between 0.76 and 0. Cleavage of the *Hpa* II-*Eco*RI (0.735–0) fragment at 0.835 defined the region coding for VP3 to be between 0.835 and 0. Thus, the mapping data can be summarized to locate VP2 between map positions 0.76 and 0, VP3 between 0.835 and 0, and VP1 between 0.835 and 0.175.

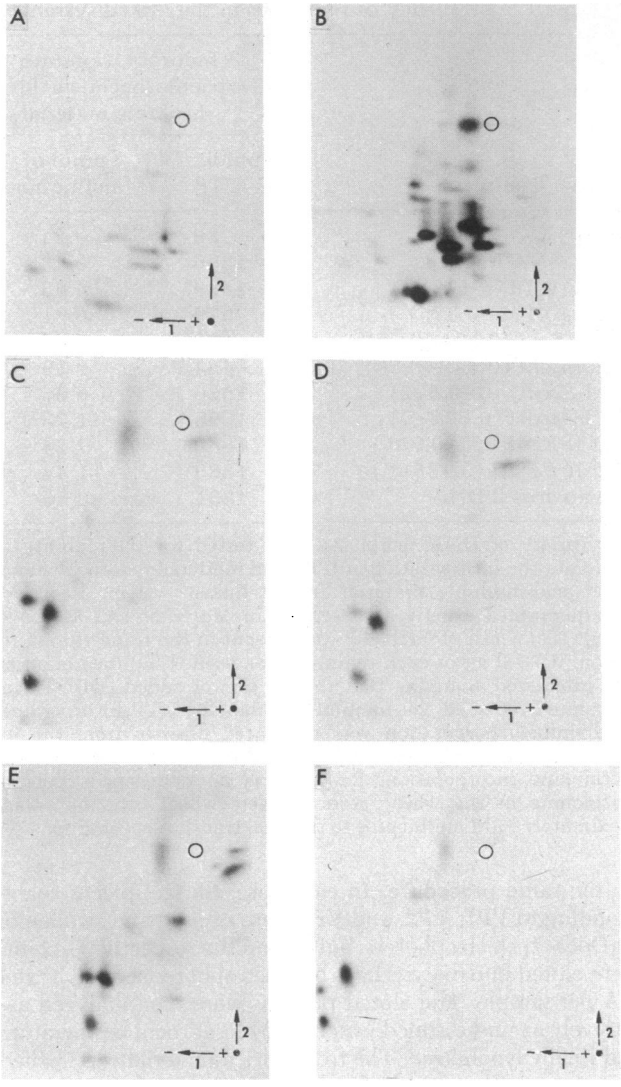

FIG. 3. Two-dimensional fingerprints of (A) putative VP1 synthesized *in vitro*, (B) VP1 from purified virions, (C) putative VP2 synthesized *in vitro*, (D) VP2 from purified virions, (E) putative VP3 synthesized *in vitro*, (F) VP3 from purified virions. The polypeptide comigrating with VP1 was isolated from the cell-free products directed by the *Hpa* I (0.76–0.175) fragment and those comigrating with VP2 and VP3 were from reactions formed with the *Hpa* II-*Eco*RI (0.735–0) fragment. Samples (1–2 μl) prepared as described in *Materials and and Methods* were subjected to ionophoresis in 0.05 M pyridine acetate buffer at pH 3.5 for 40 min (right to left), followed by ascending chromatography (bottom to top) in *sec*-butanol:propanol:isoamyl alcohol:pyridine:water (1:1:1:3:3) on cellulose-coated plates. The dried plates were autoradiographed for 10 days. The circle denotes the position of ε-dinitrophenyl-lysine marker.

The location of the regions of DNA coding for VP2 and VP3 as shown in Fig. 1A is based upon the mapping data, the close relationship between their tryptic fingerprints, and the fact that the total coding capacity (48,000 daltons of protein) of the region from 0.76 to 0 is insufficient to code for both polypeptides tandemly. It is likely that VP2 and VP3 have coincident carboxyl termini, yet this conclusion has not been demonstrated by the present data. This mapping of VP2 relative to VP3 is corroborated by deletion mutant mapping; some viable mutants of SV40 with deletions at position 0.835 lack VP2, yet have an unaltered VP3 (C. Cole, T. Landers, and P. Berg, personal communication). Further, Fey and Hirt (30) have shown that the polyoma structural proteins VP2 and VP3 share a number of common tryptic peptides.

Unfortunately, the lack of availability of enzymes that cleave SV40 DNA only between 0.835 and 0 has prevented us from assigning stricter limits on the carboxyl termini of VP2 and VP3 and the amino terminus of VP1 on the basis of mapping data alone. Therefore, we arbitrarily located the start of the sequences coding for VP1 counterclockwise from the *Eco*RI site after those sequences coding for VP2 and VP3 (Fig. 1A). Clearly, this location is based upon the assumption that there is no overlap of the sequences coding for VP2 and VP3 with those coding for VP1. An example of this type of overlap would be an out-of-frame initiation of VP1 within the terminal regions of sequences coding for VP2 and VP3. This mapping of VP1 concurs with DNA sequence data that located the initiation codon for VP1 close to 0.945 map units (31). Furthermore, our data showing that sequences coding for VP1 extend through the *Bam*HI site at 0.145 is supported by the observation that some SV40 mutants with deletions at this site are viable but synthesize a shorter VP1 (S. Goff and P. Berg, personal communication).

The fractionation of purified virions on urea–$NaDodSO_4$ gradient polyacrylamide gels established VP2 as a constant constituent of the virus. It is noteworthy that VP2 and VP3 are coded for by overlapping DNA sequences and it is assumed from the close identity of their respective tryptic fingerprints that both proteins are encoded within the same informational frame of the DNA. These results are analogous to those in the single-strand DNA phage ϕX174, wherein two polypeptides are coded for by overlapping DNA sequences in gene A (32). The significance of the relationship between VP2 and VP3 is not clear; however, our results do not support the tenet that VP2 is exclusively a precursor to VP3.

It is likely that the approach we have described will prove most useful in mapping specific eukaryotic DNA sequences which can be isolated in quantity by cloning in prokaryotic vectors.

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